A Forensic Tool Application: Distinguishing Wild from Cultured Fish

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Abstract: Effective 31 December 1990, the sale of wild-caught red drum was banned in the state of Texas. Practical enforcement of this legislation required the use of a technique that could unambiguously discriminate between wild and farm-raised fish. Fatty acid profiles were established for wild red drum from 4 major Texas bay systems as well as from 2 aquaculture ventures to determine if this technique could be a useful enforcement tool. Results indicate that fatty acid compositions, especially levels of linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), can be useful to distinguish between wild and cultured red drum. The ability to determine the origin of fish greatly enhances the enforcement of legislation designed to protect fisheries resources.

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Red drum (*Sciaenops ocellatus*) is a popular sport and commercial species of considerable economic importance to the Texas gulf coast (Matlock 1984). In the early 1980s, dwindling populations led to increasingly strict harvest regulations including reduced bag limits and a ban on commercial harvest. Continued concern for the species ultimately lead to a prohibition of the sale of wild red drum imported into the state effective 31 December 1990.

Enforcing the ban on the sale of wild red drum posed a dilemma for law enforcement officials. Specifically, there was no reliable method to discriminate between wild and commercially reared fish. Fish are accompanied to market by paper documentation indicating their origin. However, this "paper trail" is often difficult to verify. Also, the paperwork may have a "shelf-life" that extends beyond the time fish are at market. Unscrupulous individuals could easily use records from farm-raised fish to sell illegally marketed wild-caught fish. The documentation dilemma prompted Texas Parks and Wildlife Department (TPWD) biologists to investigate the efficacy of using fatty acid profiling to distinguish poached fish (i.e., wild-caught) from market-legal, commercially produced fish.

Differences in fatty acid compositions between cultured and wild fish have been reported for a variety of species (Chanmugam et al. 1986, Suzuki et al. 1986). Preliminary work by Jahncke et al. (1988) suggested that fatty acid profiling could be a useful forensic tool to distinguish wild from cultured hybrid striped bass (*Morone chrysops* X *M. saxatilis*). The technique uses the principle that "you are what you eat." Cultured fish are typically provided feeds rich in terrestrial plant protein. Some of the fatty acids in terrestrial plants occur in quantities very different from those found in aquatic environments. Fatty acids in the diet are incorporated into fish flesh and can be readily identified using gas chromatography. This paper reports the establishment of a fatty acid profile database to distinguish wild-caught from farm-raised red drum. We also report the successful application of fatty acid analysis in the prosecution of catfish poachers.

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Methods

Wild red drum comparable to market-size were collected by gill netting and by rod and reel from 4 major Texas bay systems, Matagorda, Aransas, Corpus Christi, and Upper Laguna Madre, during summer and fall 1990 and spring 1991. Commercially produced, market-ready red drum came from an outdoor pond operation in Palacios, Texas, and from an indoor recirculating operation in Bacliff, Texas. Both producers fed a commercially available feed and did not supplement with natural forage. A total of 180 wild fish and 24 commercially produced fish were used for this project. To simulate market conditions, whole fish were transported on ice to the TPWD's A. E. Wood State Fish Hatchery in San Marcos, Texas. Fish were stored on ice until processed: fillets were then stored at −80° C. Age was determined by otolith sectioning, and maturity state was assigned to all red drum used in the study.

Fish from the same season and bay were grouped into a total of 44 composites of 4 to 6 fish based on total length. Approximately 2 g of muscle tissue from each fish were taken from midway along the length of a partially thawed fillet, pooled with samples from other fish within their composite, and homogenized. Lipids were extracted from duplicate 2-g portions of this composited homogenate and methylated according to Villarreal (1992).

Approximately 0.5 μl of the fatty acid methyl esters were injected into a Varian Model 3700 gas chromatograph fitted with a 30 m x 0.25 mm ID x 0.25 μm Carbowax capillary column (Alltech, Houston, Texas). Oven temperature was held for 3 minutes at 195° C and then ramped 6° C/minute to 225° C, with a total run time of about 30 minutes. The split ratio was set at 1:100 and helium was the carrier and makeup gas with flow rates of 1 ml/minute and 30 ml/minute, respectively. Relative retention times of primary standards (Nucheck Prep; Elysian, Minn.) and the secondary standard (cod liver oil) were used for fatty acid identification. Peaks from the secondary standard were established using a previously published chromatogram (Christie 1990) and relative retention times from the primary standard.

Results

Otolith sectioning revealed that the wild red drum were primarily age 1 and 2, with ages 0 to 4 represented; all were sexually immature. Fourteen fatty acids were identified from the major peaks of chromatograms, with 4 demonstrating a consistent and distinct difference between wild and cultured red drum composite samples (Table 1). These diagnostic fatty acids were linoleic (18:2n-6), arachidonic (20:4n-6), adrenic (22:4n-6), and docosapentaenoic acid (22:5n-6). Linoleic acid was at least 4 times higher in cultured red drum than in wild red drum. Arachidonic acid was 2 to 12 times higher in wild red drum than in cultured red drum. Analysis of variance revealed that linoleic and arachidonic acids levels were significantly different ($P < 0.05$) between the wild and farm-raised red drum and thusly appear to have the greatest discriminatory power. Adrenic and docosapentaenoic acids were also significantly different between wild and cultured red drum.

Discussion

The database demonstrates the efficacy of using fatty acid composition to distinguish wild from commercially produced red drum. The use of diagnostic fatty

Table 1. A comparison of the ranges of the diagnostic fatty acid levels found in wild versus cultured red drum. Data are presented as weight percent of total fatty acids.

Red drum	Linoleic acid 18:2n-6	Arachidonic acid 20:4n-6	Adrenic acid 22:4n-6	Docosapentaenoic acid 22:5n-6
Wild	0.92–2.54	4.45–12.01	0.99–2.60	1.26–5.39
Cultured	9.62–11.51	0.85–2.29	0.16–0.32	0.20–0.60

acid profiles can provide a powerful tool to support the existing legislation and protection of the resource.

A Blind Test with a Twist

A ''blind test'' of the procedure was conducted in November 1991. Six individually tagged, whole red drum were delivered to the laboratory for analysis. Fatty acid profile analysis indicated that 3 of the fish had fatty acid compositions consistent with wild red drum. The remaining 3 fish had fatty acid profiles corresponding to commercially produced fish, although the levels of linoleic acid were somewhat lower and arachidonic acid levels were slightly higher than values established from the database. We speculated that the commercially produced fish were fed a diet that included increased fish meal when compared to the fish in the database. Another possible explanation was that the diet may have been supplemented with natural forage. The warden who provided the test fish indicated that the origin of each fish was correctly determined by fatty acid analysis. A feed sample was later obtained from the commercial producer and analyzed to provide insight into the skewed levels of linoleic and arachidonic acid present in the fish. Fatty acid analysis revealed that the producer's feed was about 2% lower in linoleic acid than the feed fed to the cultured red drum in the database; however, this did not explain the higher arachidonic levels. An inquiry to the manager of the aquaculture facility confirmed our suspicions: the fish's diet were supplemented (unintentionally) with an estimated 20% natural forage base composed of ''mud minnows'' (probably *Fundulus grandis* or a *Cyprinodon* spp.) that entered his ponds through his intake water. Therefore, not only was the feed composition slightly different from feed fed to fish comprising the database, but also these fish had access to natural forage. Even with these departures from the ''norm,'' their true identity as commercially produced fish was still detected by fatty acid profile analysis.

The Mystery "Gulf of Mexico" Fish

The lab was also provided with a red drum fillet labeled ''Gulf of Mexico.'' Our assumption was that this fillet came from a wild fish submitted to supplement the database. However, the fatty acid composition of this fish was consistent with those of farmed fish. The mystery was resolved when the warden who submitted the sample clarified that the fish was indeed from the Gulf of Mexico, but it had been pen-raised on a commercial feed under an oil derrick. Once again, fatty acid profile analysis proved correct.

The Case of the Catfish Poachers

In late December 1991, our laboratory was contacted by a federal agent involved with a multi-agency investigation into the problem of catfish poaching on Lake Texoma. We were asked if we could differentiate between wild and hatchery-reared catfish. While we had not generated our own database, Chanmugam (1986) had published information on fatty acid compositions of wild and pond-raised channel catfish. We also had some known wild channel catfish previously supplied to us to generate additional data.

In mid-January 1992, we received samples of 24 fish fillets obtained by the special agent through a sting operation on Lake Texoma. We identified the species of each fish using isoelectric focusing following the methods of Fries and Harvey (1986). All fish were identified as blue catfish (*Ictalurus furcatus*), except 2 fish identified as channel catfish (*Ictalurus punctatus*). The fatty acid profiles for all but the channel catfish were consistent with profiles for wild fish. It was later learned that one of the defendants in the case had a fish farm and was permitted to sell channel catfish, but not for selling blue catfish. The case went to Federal District Court in March 1992, and the laboratory analyses were successfully entered as evidence. A jury found all defendants guilty of the charges filed against them.

The Need for Ongoing Research

While we are certainly encouraged by the results of fatty acid profiling to date, much more remains to be done. The database for red drum should be expanded to include additional bays and aquaculture facilities. Continuous advancement in the development of aquaculture feeds necessitates routine monitoring of aquaculture facilities. Additionally, law enforcement officials suspect illegal commercial harvesting of wild populations of channel, blue, and flathead catfish (*Pylodictis olivaris*), as well as white bass (*Morone chrysops*) and hybrid striped bass, necessitating the development of extensive databases for each of these species as well.

Technically Valid and Cost-Effective

In conclusion, fatty acid profiling provides an effective tool for distinguishing between wild and farmed-raised fish. Data presented here and elsewhere suggest that the method should be applicable to a wide variety of species. In addition to being practical, fatty acid profiling is relatively inexpensive. With an initial capital investment of approximately $12,000 for the gas chromatograph and integrator, samples are thereafter run for the cost of consumable supplies, estimated at $1.07 per sample. While gas chromatographs are frequently equipped with expensive autosamplers, 1 person can complete 32 samples in 3 days without this added expense.

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